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27. (Amended) A process for obtaining a mutant polymerase comprising purifying the mutant polymerase from the isolated host cell of Claim 26.

REMARKS

Claims 15-29 are pending and under consideration in the instant application. With the instant Amendment, Claims 26 and 27 are amended. A copy of pending Claims 15-29, marked up to show amendments made, is attached hereto as *Exhibit A*.

I. THE AMENDMENT OF THE CLAIMS

Claim 26 has been amended to claim "isolated" host cells. The amendment to Claim 26 is fully supported by the specification, for example, at page 8, lines 4-11.

Claim 27 has been amended to recite an active step. The amendment to Claim 27 is fully supported by the specification, for example, page 8, lines 11-20, and by the claim as originally filed. This amendment does not narrow or change the scope of the claim. No subject matter is surrendered as a result of this amendment.

As the amendments to the claims are fully supported by the specification and claims as originally filed, they do not constitute new matter. Entry thereof is therefore respectfully requested.

II. THE REJECTIONS UNDER 35 U.S.C. § 112

Claims 26 and 27 stand rejected under 35 U.S.C. § 112.

A. The Rejection of Claim 27 Under 35 U.S.C. § 112, Second Paragraph

In order to satisfy 35 U.S.C. § 112, second paragraph, a claim must particularly point out and distinctly claim the subject matter which the applicant regards as his invention. Claim 27 stands rejected as allegedly being an incomplete process and failing to recite any method steps. The rejection is obviated and/or overcome in light of the amendment to Claim 27.

Applicants have amended Claim 27 to recite the active step of "purifying." This amendment serves to correct an error in claim language, and does not narrow or change the scope of the claim. As this amendment is believed to overcome the rejection under 35 U.S.C. § 112, second paragraph, withdrawal of the rejection is requested.

B. The Rejection of Claims 26-27 Under 35 U.S.C. § 112, First Paragraph

The first paragraph of 35 U.S.C. § 112 states that the specification must contain a written description of the invention, and the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the claimed invention. Claims 26-27 stand rejected as allegedly not being enabled for host cells in whole organisms or methods *in vivo*. The rejections are obviated and/or overcome in light of the amendment to Claims 26 and 27.

Claims 26 and 27 have been amended to recite "an isolated host cell." The Examiner notes that Claims 26 and 27 are enabled for isolated host cells and methods *in vitro*. *See* Office Action mailed June 5, 2002, p. 3, para. 6, first sentence. As this amendment is therefore believed to overcome the rejections under 35 U.S.C. § 112, first paragraph, withdrawal of the rejection is requested.

III. THE REJECTIONS UNDER 35 U.S.C. § 103

Claims 15-29 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Geneseq AAW29323 (the sequence in Frey *et al.*, DE 196 11 759 A1) in view of Pisani *et al.* (1998), *Biochemistry* 37, 15005-12, Truniger *et al.* (1996), *EMBO J.*, 15(13), 3430-41, and Truniger *et al.* (1999), *J. Mol. Biol.*, 286, 57-69. Applicants respectfully traverse the rejection of any claim under 35 U.S.C. § 103(a) since the Examiner has not made a *prima facie* case. As detailed below, there is neither motivation to combine the references nor reasonable expectation of success.

A. The Legal Standard of *Prima Facie* Obviousness

The Examiner bears the initial burden of establishing a *prima facie* case of obviousness under 35 U.S.C. § 103(a). *In re Bell*, 26 U.S.P.Q.2d 1529, 1530 (Fed. Cir. 1993); MPEP § 2142. In order to establish *prima facie* obviousness, three basic criteria must be met.

First, there must be some suggestion or motivation, either in the cited references or in the art, to modify or combine the cited references. Second, the cited references must provide a reasonable expectation of successfully achieving the claimed invention. That is, they must do more than make the claimed invention merely obvious to try, or obvious to experiment with. *See In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531-32 (Fed. Cir. 1988). The teaching or suggestion to make the claimed invention, as well as the reasonable expectation of success, must come from the prior art, not Applicants' disclosure. *In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). Third, the prior art, either alone or in combination, must teach or suggest each and every limitation of the rejected claims. *See In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991); MPEP §§ 706.02(j) and 2143.

If *prima facie* obviousness is established, an applicant can rebut the *prima facie* showing of obviousness with arguments and/or evidence demonstrating the nonobviousness of the claimed invention. A *prima facie* case of obviousness may be rebutted, for example, by showing that the claimed invention exhibits a superior property or advantage that a person of ordinary skill in the art would have found surprising or unexpected. *In re Soni*, 34 U.S.P.Q.2d 1684, 1686-87 (Fed. Cir. 1995). That which would have been surprising to a person of ordinary skill in an art would not have been obvious. *Id.*

B. Claims 15-29 are Patentable Over the Cited References

Claim 15 recites a mutant polymerase wherein the tyrosine of the Y-GG/A amino acid motif of the mutant polymerase is substituted with another amino acid, and wherein the wild-type form of the mutant polymerase has at least 80% amino acid homology to SEQ ID NO:34. Claims 16-29 depend from Claim 15.

Truniger *et al.* (1999) teaches that the Y-GG/A motif located between the – and C-terminal domains is highly conserved in most DNA polymerases belonging to the eukaryotic-type superfamily. Specifically, Truniger *et al.* (1996) and Truniger *et al.* (1999) teach the enzymatic activities of wild-type and 11 mutant φ29 polymerases, with each mutant containing a single site mutation in or adjacent to the φ29 polymerase Y-GG motif. Two mutants contained a single site mutation at the tyrosine residue within the motif, substituting either phenylalanine or serine in place of tyrosine. Pisani *et al.* teaches the polymerase activity (pol) and 3'-5' exonuclease activity (exo) of 8 mutant *Sulfolobus solfataricus* (*Sso*) DNA polymerases relative to wild-type *Sso* polymerase. Two mutants in Pisani *et al.* contained a single site mutation at the tyrosine residue within the motif, substituting either phenylalanine or serine in place of tyrosine.

Neither Truniger *et al.* (1996), nor Truniger *et al.* (1999), nor Pisani *et al.* teach mutation of a polymerase having at least 80% amino acid homology to SEQ ID NO:34. While characterizations of the pol/exo ratios are made in the cited references, nothing in these references discuss the suitability of any mutations for PCR. Indeed, as noted on page 7 (3rd paragraph) of the instant specification, neither φ29 nor *Sso* polymerases are even suitable for PCR. While Geneseq AAW29323 teaches the expression of the polymerase having 99.1% homology to instant SEQ ID NO:34, it does not teach the mutation as in Claim 15.

Moreover, Claim 19 recites the mutant polymerase of Claim 15 wherein the wild-type form of the mutant polymerase is SEQ ID NO:34. Not one of the cited references, Geneseq AAW29323, Pisani *et al.*, Truniger *et al.* (1996), or Truniger *et al.* (1999), teach a mutant polymerase wherein the wild-type form is SEQ ID NO:34. The sequence taught by Geneseq AAW29323 varies from SEQ ID NO:34 by five amino acid substitutions (4 non-conserved mutations and 1 conserved mutation). Thus, the cited references do not teach or suggest each and every limitation of rejected Claim 19.

The following discussion further explains why the cited references are insufficient to form the basis of a *prima facie* obviousness rejection of Claims 15-29.

a. <u>There is No Motivation to Combine References</u>

The mere fact that references could be modified or combined does not render the resultant modification or combination obvious unless the prior art also suggests the desirability of the modification or combination. *In re Mills*, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990); MPEP § 2143.01.

The Examiner states that "Pisani *et al.* and Truniger *et al.* both taught the motivation for mutation of the Y-GG/A motif in *any polymerase* having such a motif, including the

substitution of the Tyr for a Phe or Ser (instant claims 20-23), for *improved polymerization* of the polymerases . . . ." Office Action mailed June 5, 2002, p. 6 (emphasis added). This is not true, since Truniger *et al.* (1996) teaches that replacing tyrosine with serine to form a mutant φ29 polymerase results in <1% of the polymerase activity as that of wild-type φ29 polymerase. Truniger *et al.* (1996), p. 3433 Table 1. In other words, the tyrosine to serine mutation impedes, rather than improves, polymerization in the φ29 polymerase. Truniger *et al.* (1996), p. 3437.

On page 7 (1st paragraph) of the Office Action, the Examiner states that "[o]ne of ordinary skill in the art would have been motivated to substitute the Y-GG/A motif of a polymerase having said motif, such as the polymerase taught by Geneseq AAW29323, with different amino acids for the benefits taught by Truniger *et al.*, improved polymerization." To reiterate, neither Truniger *et al.* (1996) nor Truniger *et al.* (1999) suggest that mutation of tyrosine always leads to improved polymerization, since at least the mutation of tyrosine to serine impedes polymerization. Of course, figure 5 of the instant application demonstrates that mutation of the tyrosine to serine in *Tag* polymerase results in improved PCR fidelity as compared to wild-type. It is difficult to reconcile how a tyrosine to serine mutation shown by Truniger *et al.* (1996) to impede polymerization in the φ29 polymerase would suggest or motivate one of ordinary skill in the art to perform a similar mutation in the *Tag* polymerase..

Neither Truniger *et al.* (1999) nor Pisani *et al.* describes enzymes suitable for PCR which undermines a motivation to look to these references. More importantly, there is no suggestion in the cited references to use a tyrosine mutant in particular as opposed to any of the other possible mutations to achieve improved PCR results. This is due in part to the fact that no prior art has specifically taught that the Y-GG/A motif is important for the performance of B-type DNA polymerase in PCR. Finally, while Truniger *et al.* (1999) and

Pisani *et al.* teach the pol and exo activities of mutant and wild-type φ29 and *Sso* polymerases, *there is no correlation between the changes of pol/exo ratio and the improvement of the performance of DNA polymerases in PCR*. For example, as discussed in page 4 (1st and 2nd paragraphs), the *Tag* mutant Y387H does not exhibit a change of pol/exo ratio compared to the wild-type, but it exhibits improved performance in PCR.

Accordingly, since nothing in the prior art points to the desirability of the instant tyrosine mutations for improved PCR performance, as encompassed in Claim 15, the suggestion or motivation to modify or combine the cited references has not been established.

b. <u>There Is No Reasonable Expectation of Success</u>

The Examiner must establish a reasonable expectation of success for a rejection under 35 U.S.C. § 103(a). *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531-32 (Fed. Cir. 1988). The Examiner states that one of ordinary skill in the art would have had an expectation of success to make the claimed mutant polymerases since the methods for mutation of the Y-GG/A regions of known polymerases, such as the one taught by Geneseq AAW29323, were well-known in the art as taught by Truniger *et al.* and Pisani *et al.* Office Action mailed June 5, 2002, p. 7.

First, the effects of mutating the tyrosine residue within the Y-GG/A motif, in terms of pol and exo activities, as determined by Truniger *et al.* (1996) and Pisani *et al.* do not completely correspond to the effects obtained for the *Tag* DNA polymerase. For example, a comparison of the exonucleolytic activity of mutants containing a tyrosine to serine substitution shows that mutant *Sso* polymerase had only 5% exonucleolytic activity relative to *Sso* wild-type, whereas mutant φ29 polymerase had 380% and mutant Tag polymerase 187% relative activities to their respective wild-types. *See* instant specification, Figure 1 (Tag);

Pisani *et al.*, Table 2 (*Sso*); and Truniger *et al.* (1996), Table 1 (φ29). Thus, in this example, between Pisani *et al.* and Truniger *et al.* (1996) it is not clear whether mutating tyrosine would result in lower (similar to Pisani *et al.*) or greater (similar to Truniger *et al.* (1996)) activity than wild-type in the *Tag* polymerase. And even if cited references do generally indicate a relative increase in polymerization activity of tyrosine to phenylalanine mutants, as compared to wild-types, no cited reference discusses the resultant effect that the mutation has on PCR-based fidelity, which is described for the *Tag* polymerase on pages 6-7 of the instant application. Thus, it is difficult to understand how the prior art supports any prediction regarding PCR suitability of the instant mutation, much less a suggestion that the claimed mutation would lead to an improvement.

More fundamental however, is the fact that the effects of mutating the tyrosine in the Y-GG/A is not predictable in terms of PCR performance, since neither Sso nor φ29 polymerases are suitable for PCR. Although it has been described in the prior art that the Y-GG/A motif plays a role in the coordination of the DNA polymerase activity and the exonuclease activity, the observed changes of the pol/exo ration of the prior art DNA polymerases do not strictly correlate to the changes observed for the inventive mutants of the Tag DNA polymerase, as discussed above. It has not been described that the Y-GG/A motif is important for the performance of B-type DNA polymerase in PCR. Additionally, there is no correlation between the changes of the pol/exo ratio and the improvement of the performance of DNA polymerases in PCR.

Accordingly, for reasons detailed above, there is no suggestion or motivation, either in the cited references or in the art, to modify or combine the cited references, nor is there a reasonable expectation of success of claimed subject matter.

c. **Applicants' Invention Shows Surprising Results**

"One way for [an applicant] to rebut a prima facie case of obviousness is to make a showing of 'unexpected results,' i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 34 U.S.P.Q.2d 1684, 1687 (Fed. Cir. 1995). For reasons discussed above, the Examiner has not established a *prima facie* case of obviousness under 35 U.S.C. § 103(a) against Claims 15-29. But even assuming, *arguendo*, that the cited references could establish a *prima facie* case, it is overcome by the unexpected results achieved by the inventors. Mutants as encompassed in Claim 15 show substantial differences in the characteristics measured, relative to the *Sso* and φ29 polymerases, or what could have been predicted from Pisani *et al.*, Truniger *et al.* (1996), and Truniger *et al.* (1999).

For example, the fact that the tyrosine to serine mutant *Tag* polymerase has any polymerase activity is surprising in comparison to Truniger *et al.* (1996) who teach that the equivalent mutation in φ29 polymerase *impedes* polymerase activity. Also, the exonucleolytic activity of the tyrosine to phenylalanine *Tag* mutant remains reasonably similar to that of the wild-type *Tag* polymerase; the same cannot be said for the equivalent mutations in the *Sso* and φ29 polymerases. *Compare* Instant Specification, Figure 1 (90% for mutant *Tag* polymerase) *with* Pisani *et al.*, p. 15011 Table 2 (68% for mutant *Sso* polymerase) *and* Truniger *et al.* (1996), p. 3433 Table 1 (7% for φ29 polymerase). Indeed, the instant application teaches that the tyrosine residue in the conserved motif Y-GG/A of a polymerase having at least 80% amino acid homology to SEQ ID NO:34 plays a pivotal role in PCR-based amplification, with apparently any mutation to this residue conferring some advantage in comparison to the wild-type whether it be improved fidelity (e.g., Ser or Asn

substitution), polymerization length (e.g., His substitution), or polymerization speed (e.g., His, Trp, or Phe substitution). This is not what one of skill in the art could have predicted merely on the basis of the tyrosine to serine or to phenylalanine mutations of the other polymerases in the prior art.

In summary, there is no suggestion or motivation, either in the cited references or in the art, to modify or combine the cited references, nor is there a reasonable expectation of success of claimed subject matter. But even assuming, *arguendo*, that were a prima facie case of obviousness established, the data presented in the instant application exhibit surprising and unexpected results to a person of ordinary skill in the relevant art. Applicants respectfully request the withdrawal of the rejections of Claims 15-29 under 35 U.S.C. § 103(a).

CONCLUSION

Applicants submit that Claims 15-29 satisfy all of the criteria for patentability and are in condition for allowance. An early indication of the same and passage of Claims 15-29 to issuance is therefore kindly solicited.

No fees are believed due in connection with this response. However, the Commissioner is authorized to charge all required fees, fees under 37 C.F.R. § 1.17 and all

required extension of time fees, or credit any overpayment, to Pennie & Edmonds U.S. Deposit Account No. 16-1150.

Respectfully submitted,

Date: Nov 11 2002

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EXHIBIT A
Marked Up Claims to Show Amendments Made

15. A mutant polymerase comprising a Y-GG/A amino acid motif between an N-terminal 3'-5' exonuclease domain and a C-terminal polymerase domain wherein the tyrosine of the Y-GG/A amino acid motif is substituted with another amino acid, and wherein the wild-type form of the mutant polymerase has at least 80 % amino acid homology to SEQ ID NO:34.

16. The mutant polymerase of Claim 15 that is suitable for polynucleotide amplification.

17. The mutant polymerase of Claim 15 wherein the wild-type form of the mutant polymerase is obtainable from Euryarchaea.

18. The mutant polymerase of Claim 15 wherein the wild-type form of the mutant polymerase is obtainable from *Thermococcus aggregans*.

19. The mutant polymerase of Claim 15 wherein the wild-type form of the mutant polymerase is SEQ ID NO:34.

20. The mutant polymerase of Claim 15 wherein the tyrosine of the Y-GG/A amino acid motif is substituted with an amino acid with an aromatic side chain.

21. The mutant polymerase of Claim 20 wherein the tyrosine of the Y-GG/A amino acid motif is substituted with a phenylalanine, a tryptophan or a histidine.

22. The mutant polymerase of Claim 15 wherein the tyrosine of the Y-GG/A amino acid motif is substituted with an amino acid with a a hydrophilic side chain.

23. The mutant polymerase of Claim 22 wherein the tyrosine of the Y-GG/A amino acid motif is substituted with an asparagine or a serine.

24. A DNA encoding the mutant polymerase of Claim 15.

25. A vector comprising the DNA of Claim 24.

26. (Amended) [A] An isolated host cell comprising the DNA of Claim 24 or the vector of Claim 25.

27. (Amended) A process for obtaining a mutant polymerase comprising purifying [purification of] the mutant polymerase from the isolated host cell of Claim 26.

28. A process for synthesizing nucleic acids, comprising contacting the mutant polymerase of Claim 15 with nucleotides, a primer and a polynucleotide template under conditions suitable for elongation of the primer.

29. A process for polynucleotide amplification comprising contacting the mutant polymerase of Claim 15 with nucleotides, primers and a polynucleotide template under conditions suitable for amplification of the polynucleotide.